



1761

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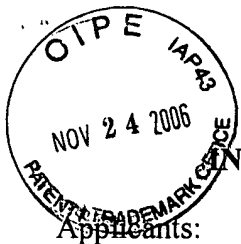
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Firm Name	Bell, Boyd & Lloyd LLC		
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Date	November 21, 2006	Reg. No.	30,142

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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SUBMISSION OF CERTIFIED COPY OF PRIORITY DOCUMENT

Applicants are respectfully enclosing the certified copy of the priority document for which priority is claimed for the above-identified application under 35 U.S.C. §119. Specifically, the document enclosed is:

<u>Document No.</u>	<u>Country</u>	<u>Date</u>
03014056.0	Europe	June 23, 2003

The Commissioner is hereby authorized to charge deposit account 02-1818 for any fees which are due and owing.

Respectfully submitted,

BELL, BOYD & LLOYD LLC

BY

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Dated: November 21, 2006

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Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

03014056.0

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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Demande no:

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Anmelder/Applicant(s)/Demandeur(s):

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

Nutritional composition

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)
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NO 7579/GF

**Patent Application
In the name of Nestec S.A.**

Title: Nutritional composition

Nutritional composition

Field of the invention

5 The present invention relates to a new and inventive nutritional composition intended for infants and/or young children, as well as to a method for strengthening natural immune defenses and to a method for promoting a healthy mental development in infants or young children by fully or partly feeding them with the afore-mentioned formula

10

Background of the invention

15 The composition of human milk serves as a valuable reference for improving infant formula. However, human milk contains living cells, hormones, active enzymes, immunoglobulins and components with unique molecular structures that cannot be replicated in infant formula. Unlike human milk, infant formula must remain stable on the shelf for up to thirty-six (36) months. These fundamental differences between human milk and infant formula often mandate differences in the composition to achieve similar clinical outcome.

20

25 The study of human milk components has stimulated many investigations into what constituents may be added to an improved infant formula. Greater knowledge of the composition of human milk affords the opportunity to design infant formulas that are closer in composition to human milk. However, it becomes increasingly apparent that infant formula can never exactly duplicate human milk. Many constituents in human milk are bioactive and because of synergies among these components, there is little reason to believe that the same compound would have the same bioactivity in infant formula. The likelihood of this possibility is further diminished when the impact of heat treatment for sterilization and long-term storage of the formula is considered.

30

35 The composition of human milk differs appreciably from that of other species and much attention has been paid to the various components. Several investigators have reported on the nucleotide content of milk from humans. Numerous publications have also discussed various lipid, oil or fat blends for use in an artificial nutritional for human infants.

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There is a need for new formulae, providing to the infant or the young child a nutritional contribution with a unique combination of protective nutrients, especially ensuring growth and metabolic patterns similar to those of breastfed infants, thus resulting in similar health characteristics in later childhood and adulthood.

5

Summary of the Invention

A first object of the present invention therefore pertains to formulae intended both for infants and young children. The formula of the invention comprises at least one LC-PUFA and at least one probiotic strain.

10

A second object of the present invention is a method for strengthening natural immune defenses of an infant or a young child consisting in fully or partly feeding said infant or child with the afore-mentioned formula.

15

A third object of the present invention pertains to a method for promoting a healthy mental development of an infant or a young child consisting in fully or partly feeding said infant or child with the afore-mentioned formula.

20

Detailed Description of the Invention

In the present specification, the following words are given a definition that must be taken into account when reading and interpreting the description, examples and claims.

25

Infant: according to the Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae, article 1.2(a), the term "infants" means children under the age of 12 months. This definition is adopted in the present specification.

30

Young Children: according to the Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae, article 1.2(b), the term "young children" means children aged between one and three years. This definition is adopted in the present specification.

35

Infant formulae: according to the Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae, article 1.2(c), the term "infant formula" means foodstuffs intended for particular nutritional use by infants during the first four to six months of life and satisfying by themselves the nutritional requirements of this

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category of persons. This definition is adopted in the present specification. It has to be understood that infants can be fed solely with infant formulae, or that the infant formula can be used by the carer as a complement of human milk. It is synonymous to the widely used expression "starter formula".

5

Follow-on formulae: according to the Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae, article 1.2(d), the term "follow-on formulae" means foodstuffs intended for particular nutritional use by infants aged over four months and constituting the principal liquid element in a progressively diversified diet of this category of persons. This definition is adopted in the present specification.

10

According to a first object of the invention, there is provided a nutritional composition for infants (including a starter composition) or young children. This composition, as already mentioned, is a unique combination of protective nutrients ensuring improved natural defenses compared to bottle-fed infants and children, characterised by an effects on flatulence, vomiting, regurgitation and morbidity, and also characterised by reduced diarrhea and improved response to vaccination.

15

The unique combination of the invention also results in promoting a healthy mental development of the infant and child partly or fully fed with the formula of the invention.

20

Furthermore, the nutritional composition comprises at least one probiotic selected to exert its beneficial effects all along the intestinal tract and support a healthy gut flora.

Of course, the composition of the formula according to the invention also supplies the infant or the young child with vitamins and minerals recognised as essential for a healthy development, as well as semi-essential nutrients which may be needed in particular conditions. These semi-essential nutrients can include taurine, nucleotides, carnitine, and/or selenium.

25

The formula consists of proteins, carbohydrates, at least one probiotic, fats including at least one LC-PUFA with vitamins and minerals in amounts necessary to provide the sole source of nutrition for healthy term infants from birth until the age of 4-6 months for infant formulae, and as the principal liquid element in a progressively diversified diet of infants aged over four months,

30

The nutritional composition according to the first object of the present invention comprises a special blend of fats, comprising at least one LC-PUFA. Fat provides about

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half of the dietary energy and constitutes the major energy stores in the bodies of infants and young children. Presently, there is growing interest in the quality of the dietary lipid supply during infancy as a major determinant of growth, visual and neural development, and long-term health. Thus, the selection of the dietary lipid supply during early life is considered to be of great importance.

Because of the small size of their stomach and their limited tolerance towards hypertonic foods, infants require a concentrated source of energy. Of the 3 nutrients supplying energy, fat provides 9 kcal per gram, i.e. more than twice the energy present in carbohydrates or proteins. Most experts recommend that fat in infant formulae supply from 30% to 55% of the total energy.

Reconstituted at a rate of 67 kcal/100 ml, the composition according to this aspect of the present invention supplies a quantity of fat which is close to the average value found in breast milk.

Preferably, vegetable fats, and eventually a LC-PUFA oil mixture (fish oil & Mortierella alpina oil blend, for example) only are used in the manufacture of the composition. However, whey and skim milk contain naturally some traces of milk fat, explaining a very small percentage of milk fat in the formula.

Fatty acid composition of the diet determines fatty acid composition of all tissues, including storage tissues. The fat mixture in the infant formula therefore has an overall fatty acid composition as close as possible to that of human milk, in order to ensure similar membrane plasticity and same mobilization of energy in case of increased needs. The fat mixture supplies docosahexaenoic acid and arachidonic acid, in addition to essential fatty acids (linoleic and α -linolenic acids), as well as adequate quantities of the following fatty acids:

- Oleic acid (C18:1 n7): recent data have shown that monounsaturated fatty acids (oleic acid being the main one) decrease total cholesterol and LDL-cholesterol concentrations.
- Palmitic acid (C16:0): it has been suggested that palmitic acid may be preferentially used for the synthesis of lung surfactant and thus would intervene in optimal development of respiratory function.
- Lauric and myristic acids (C12:0 and C14:0): they are medium chain saturated fatty acids, are easy to absorb, but need to be supplied at low levels. The EC Directive has proposed a maximum of 15% lauric acid and myristic acid in fat mixtures used for infant feeding. The present composition fat mixture preferably stays well below the maximal values allowed, for example between 11 and 12%.

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The fat fraction of the present formula also comprises an LC-PUFA oil mix.

Human milk contains docosahexaenoic acid (DHA) and arachidonic acid (ARA) and thus breast-feeding provides infants with preformed LC-PUFAs. The DHA content of human milk varies considerably within populations and is strongly influenced by maternal diet. Globally, the DHA content of milk from mothers consuming Western diets ranges from 0.1 to 0.4%, with a mean of 0.25%, whereas in mothers consuming non-Western diets, the DHA content of milk is greater, ranging from 0.1 to 1.4%, with a mean of 0.6%. However, amounts of 0.2 to 0.3% are generally accepted as representative. The ARA content of human milk is less influenced by the diet than DHA. Globally, the ARA content of human milk from mothers consuming Western diets ranges from 0.2 to 0.7%, with a mean of 0.45%, whereas in mothers consuming non-Western diets, the ARA content ranges from 0.4 to 1.2%, with a mean of 0.6%. Both DHA and ARA levels are influenced by the duration of lactation and tend to decrease from colostrum to transitional and mature milk.

The LC-PUFA is at least represented by DHA, preferably provided by a natural fish oil that supplies with a DHA/EPA (EPA: eicosapentaenoic acid) ratio > 4, similar to human milk. Together with DHA, the composition may provide a source of ARA, for example from fungal origin (*Mortierella alpina*, belonging to the American Type Culture Collection).

Arachidonic acid is widely distributed in all cell membranes; it is the major LC-PUFA in most peripheral tissues (e. g. heart, liver) and it is present in larger amounts in nervous tissue. It is also the precursor of biological substances known collectively as eicosanoids: prostaglandins, leukotrienes and thromboxanes which have a role in immunoregulation, in inflammatory processes and muscle contraction. Arachidonic acid is considered as being important for optimal growth, as a significant correlation has been found between plasma arachidonic acid levels and infant body growth.

In contrast to ARA, DHA accounts only for a small percentage of the fatty acid content in most tissues, except in neuronal tissues, such as the retina and the brain.

In the retina, it is concentrated in the specialized membranes of the photoreceptor outer segments that are dynamic structures whose components are renewed daily, and represents up to 50% of the fatty acids of the main phospholipids. Animals with low DHA retinal levels present with abnormal electroretinograms.

In the brain, the total amount of DHA increases dramatically during the brain growth spurt, both because of the growth of brain in size (from 100 g at the beginning of the third trimester of pregnancy to about 1100 g 18 months postnatally), but also because there is an increase in the relative DHA content, which has been calculated to increase

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approximately 35 mg per week from the beginning of the last trimester of pregnancy till the end of the first year of life.

5 As there is competition between fatty acids of the n-3 and n-6 pathways with respect to elongation and desaturation, as well as for incorporation into phospholipids and conversion to eicosanoids, we have balanced the fat in infant formulae with respect to n-6 and n-3 fatty acids. Supplementation of infant formula with only alpha-linolenic acid as a source of n-3 fatty acids, even in the recommended balance with linoleic acid, does not support DHA status equivalent to that of breast-fed infants. Indeed, numerous studies have demonstrated higher levels of DHA in circulating pools of lipids: plasma phospholipids, red blood cell lipids, red blood cell phospholipids, red blood cell phosphatidylethanolamine in breast-fed compared to formula-fed infants.

10 The arachidonic acid status in most cases is not affected and similar to that of breastfed infants. Numerous studies have shown that it is possible to achieve DHA levels in the various blood pools of formula-fed infants similar to or even higher than those of breast-fed infants by supplementing the formula with DHA. High amounts of DHA alone, or use of DHA sources providing high levels of EPA, a fatty acid precursor of DHA, may however lead to depletion of the arachidonic status. Thus, DHA in the formula according to the present invention is provided by a low EPA fish oil at a level which has been shown to achieve DHA levels in the various blood pools of formula-fed infants similar to those of breast-fed infants.

15 20

25 The composition according to the invention comprises at least one LC-PUFA. The preferred LC-PUFA is DHA, which can be the sole added LC-PUFA. In another aspect of the invention, both DHA and ARA are added into the formula.

30 According to the present invention, the infant formula comprises at least one probiotic, in order to offer all infants, whatever their mode of delivery or their hygienic environment, the advantages of a protective intestinal flora.

35 A preferred probiotic consist in Bifidobacteria, which as a whole are safe and are L (+) lactic acid producing cultures. A particularly preferred probiotic is *Bifidobacterium lactis*, first sold by Christian Hansen under the name Bb12. *B. lactis* is a Gram-positive, catalase negative strain, producing only L(+) lactic acid. The strain *Bifidobacterium lactis* (BL) has been selected to be added to the present formulae mostly because of its resistance towards acid and bile salts, and its survival not only in products with a short

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shelf life such as chilled dairy products, but also in powder milks with a longer shelf life such as infant formulae.

5 *B. lactis* appearance in the faeces of adults during ingestion of the probiotic has been confirmed by a specific PCR-ELISA method. In infants, 2 studies have been looking to the presence of *B. lactis* in the stools of infants fed a formula enriched with *B. lactis*, either by classical microscopical examination or by the more specific method of Random Amplified Polymorphic DNA (RAPD)-PCR method. It was possible to estimate the percentage of recovery of *B. lactis* in the stools to be in the range of 25-10 30%, a value that has been found for other strains of bifidobacteria and is significantly higher than other strains of probiotics.

15 Another preferred probiotic consists in a Streptococcus, particularly *Streptococcus thermophilus* provided under the name TH4 by Chr. Hansen, Denmark. Both *Bifidobacterium lactis* and *Streptococcus thermophilus* have been given a GRAS status (generally recognised as safe) by the USFDA (United States Food and Drug Administration) for use in formulas intended for children over 4 months of age, and the same USFDA has authorized marketing of a starter formula enriched with *Bifidobacterium lactis*.

20 Other preferred probiotics are Lactobacillus GG (ATCC 53103) and Bifidobacterium longum.

25 The probiotics according to the present aspect of the invention are preferably present in an amount of 10^6 to 10^9 cfu/ grams of dry product, preferably 10^6 to 10^8 cfu/g, and even more preferably 2×10^7 cfu/grams of dry product.

30 The composition according to the present invention comprises at least one probiotic. Preferably, such probiotic is a Bifidobacteria, and more preferably is *Bifidobacterium lactis*. It may also be a Streptococcus, the preferred one being *Streptococcus thermophilus*. In a very preferred embodiment of the invention, infant and starter formulae comprise *Bifidobacterium lactis* and follow-on formulae comprise both *Streptococcus thermophilus* and *Bifidobacterium lactis*.

35 Dietary protein provides the essential amino acids necessary for protein synthesis and growth and protein quality is as important as protein quantity.

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Until now, in order to supply enough of the essential amino acids, cow's milk-based infant formulae needed a protein content significantly higher than that of the reference human milk. However, if the amino acid pattern of a cow's milk-based infant formula is closer to that of human milk, the protein content of such a formula can be lowered to resemble that of the reference. A new protein mixture of unique amino acid composition allowing the adaptation of the quantity of protein to a level closer to the average content of human milk has been developed according to an aspect of the present invention.

The protein content of regular whey-adapted formulae ranges from 2.1 to 2.6 g per 100 kcal, whereas the content of human milk ranges from 1.4 to 1.8 g per 100 kcal. Excess protein intake may induce metabolic stress on infant organs that have not fully developed.

Following paediatric recommendations for lowering protein density of infant formulae, clinical trials in infants fed formulae containing protein densities between 1.6 and 2.0 g / 100 kcal have been reported. However, these attempts to lower protein content in a formula using traditional cow's milk protein sources or mixing the currently available fractions – casein and whey –, although demonstrating the principle was conceivable, failed to reproduce all the indices of human milk protein metabolism or to ensure the satisfactory growth of infants.

For instance, results have shown a global plasma amino acid pattern different to that of breast-fed infants, depressed plasma tryptophan levels, elevated plasma threonine levels, delay in growth, and higher energy intake suggesting an increased fat deposition which may be responsible for obesity in later life.

Cow's milk "whey protein" is a mixture of several proteins, which all have a different amino acid profile, and of the non-protein nitrogen (NPN) fraction. The caseino-glyco-macropptide (CGMP) is a protein fraction that is found in this fraction. It comes from the kappa-casein that is split up by proteolytic cleavage into 2/3 para-kappa-casein, an insoluble fraction that remains in the casein fraction and 1/3 CGMP, a soluble fraction that is found in the whey fraction.

An original fractionation process of whey proteins has been developed and is explained in EP 880902; this process allows the removal of practically all the caseino-glyco-macropptide (a fraction rich in threonine and poor in tryptophan) from bovine whey thereby increasing the alpha-lactalbumin proportion (a fraction very rich in tryptophan).

By combining this modified sweet whey fraction with skim milk, and with the addition of some free L-histidine and L-arginine (in order to reach the minimum amounts of

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these amino acids required by EC Directive), the formulation according to the invention has an amino acid profile much closer to that of human milk, characterised in particular by comparable tryptophan and threonine levels, allowing the adaptation of its protein content to that of human milk.

5

Nutritional value of the new protein mixture used in the manufacture of the protein profile according to the invention has been measured in rats.

The results show (see table 1) that this formulation has a Protein Efficiency Ratio (PER), a nitrogen digestibility, a Biological Value (BV), and a Net Protein Utilisation (NPU) comparable to standard whey-adapted formulae.

10

Table 1

Nutritional parameters	Casein	standard whey-adapted formula	formulation of the invention
PER	1.36	2.49	2.70
Relative PER (casein = 100%)	100.0	182.8	198.3
Digestibility (%)	96.7	92.8	91.4
BV	0.88	0.96	0.96
NPU (%)	85.4	88.8	87.5

Moreover, rats fed on the formula according to the invention showed significant lower plasma threonine levels and increased plasma tryptophan levels, compared to rats fed on standard whey-adapted formulae.

15

The protein profile of the composition according to the present invention, with high protein efficiency, is a very well adapted infant formula with a protein content closer to that of human milk. With a protein content being at maximum of 2g/100 kcal, preferably 1.85, most preferably between 1.8 and 1.85 g/100 kcal, it is in the lower part of the range of the most recent paediatric recommendations for infant formulae. Moreover, this level is in line with recent data assessing protein requirements during early life, which has shown that recommendations for optimal protein intakes are lower than they have been reported in the past.

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To ensure optimal protein synthesis, and therefore optimal growth, essential and semi-essential (i.e. essential only during infancy) amino acids need to be supplied in the same quantities as in human milk.

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5 The formulation according to the invention is preferably either whey enriched (casein / whey ratio set around 40/60 or lower, such as 45/55), either whey predominant (casein / whey ratio preferably set at 30/70 or even more, such as 20/80). Together with the with the unique protein mixture and casein / whey ratio, the amino acid profile of the composition according to the invention is comparable to that of human milk (see table 2).

Table 2

Amino acid (g / 16 g N)	Human milk			invention (representative values)
	mean	lowest value	highest value	
Isoleucine *	6.4	5.7	6.8	5.8
Leucine *	11.5	11.0	11.9	11.9
Lysine *	7.9	7.4	8.4	10.0
Methionine *	1.7	1.3	2.1	2.5
Cystine **	2.3	1.7	2.9	2.4
Phenylalanine*	4.6	4.2	5.1	4.6
Tyrosine **	4.7	3.3	6.3	4.0
Threonine *	5.6	5.3	6.6	5.4
Tryptophan *	2.3	1.8	2.6	2.1
Valine *	6.8	5.9	8.0	5.9
Arginine **	4.2	3.5	4.9	4.5
Histidine **	2.8	2.4	3.8	2.5
Alanine	4.8	4.5	5.3	5.1
Aspartic acid	10.4	10.1	10.8	11.1
Glutamic acid	19.6	17.6	22.7	19.7
Glycine	3.2	2.8	3.6	2.7
Proline	10.2	8.9	11.2	7.8
Serine	5.6	5.0	5.9	5.3

10

All values corrected to 40% NH₃

* essential amino acids ** semi-essential amino acids

Preferably, the proteins are non-hydrolysed proteins.

15

The sole source of carbohydrates of the composition according to the present invention is lactose. In less preferred embodiments of the invention, however, other sources of carbohydrates, such as for example saccharose, maltodextrins, and/or starch can be used together with lactose, in various ratios.

20

Carbohydrates constitute an important source of energy in the diet of the newborn infant. Lactose is the natural carbohydrate in human milk. Most infants in good health can digest lactose adequately.

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5 Lactose is associated with stool acidity and an intestinal flora (preponderance of lactobacilli and bifidobacteria) which may be important in suppressing the growth of undesirable bacteria in the intestine of breast-fed infants. Moreover, lactose has been shown to enhance absorption and retention of calcium and probably other minerals. In a recent study, it has been shown that calcium absorption is 10% greater from a lactose-containing formula compared with the same formula in which the lactose was replaced by glucose polymers.

10 The formula according to the first object of the invention may also supply semi-essential nutrients which may be needed in particular conditions (e. g. taurine, nucleotides, carnitine, selenium).

15 Taurine is a free amino acid, which is not used to build up protein molecules. It has been shown to be involved in many physiological functions, e.g., as a trophic factor in the development of the central nervous system, maintaining the structural integrity of the membrane, regulating calcium homeostasis, as an osmolyte, a neuromodulator, and a neurotransmitter. It also conjugates with bile acids to form bile salts (essential for micelle formation and fat absorption).

20 Nucleotides are non protein nitrogen compounds which contain three characteristic components: a nitrogenous base, a sugar (ribose or deoxy-ribose), and one or more phosphate groups. Total nucleotide content in human milk represents 2 to 5% of the non-protein nitrogen. Cow's milk contains lower concentrations of nucleotides than human milk and its nucleotide profile differs markedly from that of human milk. Addition of nucleotides in the present infant formula follows the physiological pattern of nucleotides levels in human milk, with a predominance of easily metabolised pyrimidines over less desirable purines: addition of nucleotides to the infant formula is safe. The levels of addition are within the range allowed by the European Union Scientific Committee for Food and the European Directive.

30 Carnitine is a particular nitrogenous compound, which belongs to a group of food factors known as vitamin-like nutrients, performs a crucial role in the energy supply of tissues during foetal life and in the neonatal period by facilitating the transport of long chain fatty acids into the mitochondria where beta-oxidation occurs. Fatty acids are indeed not able to pass in free form through the mitochondrial wall; the transfer into the mitochondria is governed by at least three enzymatic systems, namely carnitine –

palmitoyl transferases I and II and carnitine -translocase, in which carnitine participates. Thus, carnitine is required for proper lipid oxidation and carnitine deficiency or low carnitine intake can lead to impaired fat utilisation and altered lipid metabolism. Carnitine has also a role in other metabolic processes, such as ketogenesis, lypolysis, and the maintenance of thermogenesis and nitrogen metabolism. Moreover, carnitine has been shown to improve utilisation of medium chain triglycerides in infants.

New-borns have relatively low carnitine reserves and a very low activity of the enzyme catalysing the last step in the carnitine synthesis. Thus new-borns are particularly at risk of becoming carnitine-deficient in the absence of an adequate supply of exogenous carnitine. Carnitine is preferably added to infant formulae, in order to reach a level close to that of human milk.

The product according to the first object of the invention may be in powder form or as a ready to drink solution.

In the case of a powder formulae, the following feeding table may be used as a guide. However, the quantities may be changed according to medical advice. The introduction of cow's milk or any other infant formula must be carried out under medical supervision. The standard reconstitution of the formula according to the invention is 12.9%, i.e. 12.9 g powder for 90 mL of water, which gives a caloric density of 67 kcal/100mL.

Table 4

Age of infant	quantity per feed		No. of feeds per day	
	Previously boiled water (mL)	number. of measuring scoops	Formula	Others
1 st and 2 nd weeks	90	3	6	-
3 rd and 4 th weeks	120	4	5	-
2 nd month	150	5	5	-
3 rd and 4 th months	180	6	5	-
5 th and 6 th months	210	7	5	-
from the 7 th month onwards	210	7	4-3	1-2

In the case of a ready-to-drink solution, a special care needs to be given so that the probiotics do not enter in contact with the liquid formula accidentally. Preferably, the probiotics are stored in powder apart from the liquid formula, and is incorporated and homogenised into the liquid formula just before consumption, i.e up to two hours before consumption.

5 A second object of the present invention is a method for strengthening natural immune defenses of an infant or a young child consisting in fully or partly feeding said infant or child with a formula as described above, i.e. comprising at least one probiotic and at least one LC-PUFA.

10 Intestinal mucosa is one important location for the immune system and the gastro-intestinal microflora plays a dominant role in the development of the gut-associated lymphoid tissue (GALT). This highly organized immune system consists of lymphoid follicles that can be either isolated or grouped in Peyer's patches present in the deep part of the mucosa and the submucosa of the small intestine. GALT has the capacity to discriminate between pathogenic microorganisms to which it responds dynamically, and the vast array of dietary antigens and commensal microbial flora to which it remains tolerant. Probiotics interact with the immune system at many levels, including cytokine
15 production, mononuclear cells proliferation, macrophage phagocytosis and killing, modulation of autoimmunity, and immunity to bacterial and protozoan pathogens.

These immunological properties are strain-specific. *Bifidobacterium lactis* has been shown to positively influence mucosal immunity: in adult subjects, *B. lactis* enhances stimulation of phagocytosis by peripheral blood lymphocytes whereas in infants, *B. lactis* enhances secretion of faecal IgA, immunoglobulins which play an important role
20 in pathogens elimination.

More important, this immune stimulation results in a clear health benefit, i.e. reduction of the risk of diarrhoea in infants at high risk of contamination as hospital environment and in the more usual conditions of day-care centres. A similar trend was found recently
25 in a study comparing a starter whey hydrolysed formula with different protein levels and *B. lactis* addition. Salivary rotavirus-specific IgA titres are a good indicator of rotavirus infections. Whereas they are not detected in healthy neonates, they are increased in infected infants. Infants and children fed a *B. lactis* enriched formula have less often an increase in their salivary anti-rotavirus titres when exposed to a contaminated environment, supporting the hypothesis that *B. lactis* supplementation
30 protects against rotavirus infection.

Inflammation (usually characterised by redness, swelling, heat and pain) is a normal, immediate response of the body to infection. It is part of the normal, innate immune system. A too strong immune reaction may thus lead to excessive inflammatory
35 reaction. Allergy is also the result of an exacerbated immune reaction due to inappropriate recognition and response to antigens. Appropriate stimulation of the

immune system should therefore result in adequate protective mucosal immunity without excessive inflammation and develop systemic oral tolerance,

In the newborn, the pattern of immune response is skewed towards Th-2 type of response, leading to allergic reactions, and will evolve during postnatal maturation towards a balanced Th-1/Th-2 response.

The intestinal flora counterbalances Th2 activity and affects the development of many other immune parameters. Differences in intestinal flora composition exist between infants developing allergy and healthy infants: infants with atopic dermatitis are less frequently colonized by bifidobacteria as compared to healthy ones. Probiotics are therefore considered as potential modulators of the allergic reactions. But similarly to the immune protection, this activity is strain-specific. The anti-inflammatory properties of *B. lactis* have been shown first in *in vitro* models of cell cultures and confirmed in highly sensitized infants who did not react to feeding with an extensively hydrolyzed infant formula. In such infants, *B. lactis* reduces the symptoms of atopic dermatitis. Further, supplementation with *B. lactis* prevents the increase in the numbers of bacteroides and *E. coli* during weaning, and high numbers of bacteroides and *E. coli* are associated with the extent of atopic sensitization in infants with atopic eczema.

The so-called "hygiene hypothesis" suggests that the increase in allergic disease may be due to a lack of stimulation of the immune system by microbial exposure and resulting prolongation of the immature neonatal pattern of immune response well into the first years of life. Since the pattern of response associated with the first encounter with an antigen is likely to be programmed into long-term immunological memory, an innocuous early life exposure as realized by selected probiotics such as *B. lactis* may further contribute to an optimal health status later in life.

The quantity and quality of dietary lipids and their metabolism are of major importance for the growth, body composition, development and long-term health of children, both in health and disease. Lipids are the major source of energy in early childhood and supply essential lipid-soluble vitamins and polyunsaturated fatty acids that are required in relatively high amounts during early growth. Lipids affect the composition of membrane structures, and modulate membrane functions as well as the functional development of the central nervous system. Some LC-PUFAs serve as precursors for bioactive lipid mediators, including prostaglandins, thromboxanes and leukotrienes, which are powerful regulators of numerous cell functions such as thrombocyte aggregation, inflammatory reactions and immune functions.

The fatty acid composition of inflammatory and immune cells is sensitive to change according to the fatty acid composition of the diet. In particular, the proportion of

different types of PUFAs in these cells is readily changed, and this provides a link between dietary PUFA intake, inflammation, and immunity. The n-6 PUFA arachidonic acid is the precursor of prostaglandins, leukotrienes, and related compounds, which have important roles in inflammation and in the regulation of immunity. Among other compounds, lipids, especially n-3 polyunsaturated fatty acids, were shown to influence the immune response. Fish oil contains the n-3 PUFAs EPA and DHA. Feeding fish oil results in partial replacement of ARA in cell membranes by EPA. This leads to decreased production of ARA-derived mediators. In addition, EPA is a substrate for cyclooxygenase and lipoxygenase and gives rise to mediators that often have different biological actions or potencies than those formed from ARA. Animal studies have shown that dietary fish oil results in altered lymphocyte function and in suppressed production of pro-inflammatory cytokines by macrophages. Supplementation of the diet of healthy human volunteers with fish oil-derived n-3 PUFA results in decreased monocyte and neutrophil chemotaxis and decreased production of pro-inflammatory cytokines.

Cyclooxygenase and lipoxygenase catalyse the synthesis of eicosanoids from LCPUFA precursors (ARA, EPA). Eicosanoids are C-20 PUFA derivatives that include prostaglandins, thromboxanes, and leukotrienes. Among their ubiquitous biological effects such as on the immune cell functions, they are involved in the normal regulation of gastric secretion and gastric motility, as well as in gastric mucosal defence.

n-3 Fatty acids influence inflammatory cell activation processes from signal transduction to protein expression even involving effects at the genomic level. n-3 Fatty acid-mediated mechanisms decreased cytokine-induced adhesion molecule expression, thereby reducing inflammatory leucocyte-endothelium interactions and modified lipid mediator synthesis, thus influencing the trans-endothelial migration of leucocytes and leucocyte trafficking in general. Even the metabolic repertoire of specific immunocompetent cells such as cytokine release or proliferation is modified by n-3 fatty acids.

PUFAs have implications on T-cell function for the neonates. Infant survival depends on the ability to respond effectively and appropriately to environmental challenges. Infants are born with a degree of immunological immaturity that renders them susceptible to infection and abnormal dietary responses (allergies). T-lymphocyte function is poorly developed at birth. The reduced ability of infants to respond to mitogens may be the result of the low number of CD45RO+ (memory/antigen-primed) T cells or their limited ability to produce cytokines, particularly interferon- γ and interleukins IL-4, and IL-10. There have been many important changes in optimising

breast milk substitutes for infants; however, few have been directed at replacing factors in breast milk that convey immune benefits.

5 Compared with standard formula, feeding a formula containing DHA and ARA increases the proportion of antigen mature (CD45RO+) CD4+ cells, improves IL-10 production, and reduces IL-2 production to levels not different from those of human milk-fed infants.

After the oral mucosa, the intestinal epithelium and its associated gut-associated lymphoid tissue are the primary targets of dietary components.

10 Plasma membranes of many cell types contain domains enriched in specific lipids (saturated fatty acids, sphingolipids) and cholesterol, called lipid rafts. These serve as entry sites for several receptor-mediated signalling events by stabilising receptor/kinase interactions, suggesting an involvement in the initiation of signalling cascades. Cross-linking of surface receptors in hematopoietic cells results in the enrichment of these
15 receptors in the rafts along with other downstream signalling molecules. A possible explanation of how signal is transduced through the plasma membrane has arisen from the concept of rafts. From the study of cellular responses in the plasma membrane which enrich members of the Src-family tyrosine kinase, rafts can function as centres of signal transduction by forming patches. Under physiological conditions, these elements
20 synergise to successfully transduce a signal at the plasma membrane.

In T lymphocytes, key T cell antigen receptor (TCR) signalling molecules associate with rafts, disrupting raft-association of certain of these abrogates TCR signalling. The TCR itself associates with lipid rafts, and TCR cross-linking causes aggregation of raft-associated proteins. Furthermore, raft aggregation promotes tyrosine phosphorylation
25 and recruitment of signalling proteins, but excludes the tyrosine phosphatase CD45. Rafts are thus suggested to be important in controlling appropriate protein interactions in hematopoietic cells, and aggregation of rafts following receptor ligation may be a general mechanism for promoting immune cell signalling. Although not wishing to be bound by theory, we believe that the rafts, rich in saturated fatty acids, are influenced by
30 dietary LC-PUFAs explaining part of their biological effects on immune function.

A clear effect of LC-PUFAs or their precursors have been demonstrated on functions such as systemic immunity or lipid and carbohydrate metabolism, although most of them have been done on adult humans or animals.

35 Dietary LC-PUFAs are absorbed and incorporated to the membranes of the enterocytes. They appear to modulate the local inflammatory response and promote intestinal repair after stress. Therefore, dietary LC-PUFAs improve the repair of small intestine, for

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example in individuals that have been previously malnourished. The possible mechanisms whereby LC-PUFAs can affect the inflammatory cascade are multiple. Nonetheless, the role of LC-PUFAs in specifically modulating gut inflammation remains unclear.

5

Several reports suggest up-regulation of the non-specific barrier function by the products of PUFAs. Thus eicosanoids, particularly those derived from ARA, would affect intestinal secretion, mucus secretion and density of surfactant in mucus, phospholipid synthesis and provide cytoprotection to the GI mucosa. It has also been suggested that intestinal glycosyltransferases are modulated by the global unsaturation index of the fatty acids in the diet, while occludin (major component of the tight junction complex) expression would be up-regulated by gamma-linolenic acid (18:3n-6) and eicosapentaenoic acids and down-regulated by AA and linoleic acid (18:2n-6).

10

Finally, PUFAs and LC-PUFAs might be able to modulate the composition of the intestinal flora. Linoleic and alpha-linolenic acids suppress the proliferation of *Staphylococcus aureus*. Similarly, relatively high concentrations, although still in the physiological range, of free linoleic, gamma-linolenic, arachidonic, alpha-linolenic, and docosahexaenoic acids inhibit growth and mucus adhesion of *Lactobacillus GG*, *casei* and *bulgaricus*. Moreover, milder concentrations of gamma-linolenic acid and ARA promote growth and mucus adhesion of *L. casei*.

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Furthermore, the adhesion of those bacteria to Caco-2 cells grown in PUFA-containing media is modulated by the type and concentration of LC-PUFAs. Given that the ability to adhere to the intestinal surfaces appears to be important for the functionality of the probiotics and for the virulence of the pathogenic bacteria, we believe that the supplementation with LC-PUFAs affects the efficacy of the probiotic and the invasive capacity of the pathogens.

25

Although not wishing to be bound by theory, we believe that the beneficial effect of probiotics are improved by their combination with LC-PUFAs, and that LC-PUFAs promote the actions of probiotics. Therefore, a formula according to the invention exploits the synergic effect of these two components.

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Said synergic effect has beneficial actions on many visible parameters, especially in infants. Among them, a reduced diarrhoea and in some cases reduced flatulence,

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We have also paid attention to morbidity, that is to say to episodes of fever, respiratory tract infections. The first results of the study indicate a decrease in morbidity although full results are not available yet.

- 5 Further, antibody titres to vaccinations (Diphtheria, Tetanus, Pertussis, Haemophilus influenzae type b, Hepatitis B) have been measured. The first results of the study indicate an increase in antibody titres, although full results are not available yet.

- 10 According to a third object of the present invention, there is provided a method for promoting a healthy mental development of an infant or a young child consisting in fully or partly feeding said infant or child with a formula as described above, i.e. comprising at least one probiotic and at least one LC-PUFA.

- 15 LC-PUFA supplementation of infant formulae has been advocated mainly with the idea that it would improve visual function and neuro-development. Indeed the results in term infants are highly controversial. Whereas positive outcomes have sometimes been observed in small scale studies, they could not be confirmed in larger studies.

- Using the Teller acuity card procedure or the Visual Evoked Potential (VEP) acuity, observational studies in general show better retinal function in breast-fed infants than in infants fed formula without DHA and/or DHA & ARA.

- 20 Several standardized procedures of global neurodevelopment are used in studies of LC-PUFA status: the Bayley Scales of Infant Development (providing among other tools the Bayley Psychomotor Development Index and the Bayley Mental Developmental Index), the Brunet-Lezine Test, the MacArthur Communicative Development Inventories, as well as more specific unstandardized test such as the problem-solving ability.

25 Several studies show a positive effect of adding DHA or DHA & AA to the formula on cognitive development.

Examples

- 30 The following examples are illustrative of some of the products and methods of making the same falling within the scope of the present invention. They are not to be considered in any way limitative of the invention. Changes and modifications can be made with respect to the invention. That is, the skilled person will recognise many variations in
- 35 these examples to cover a wide range of formulas, ingredients, processing, and mixtures to rationally adjust the naturally occurring levels of the compounds of the invention for a variety of applications.

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Example 1 : Preferred formula

Nutrient	per 100kcal	per litre
Energy (kcal)	100	670
Protein (g)	1.83	12.3
Fat (g)	5.3	35.7
Linoleic acid (g)	0.79	5.3
α -Linolenic acid (mg)	101	675
Lactose (g)	11.2	74.7
Minerals (g)	0.37	2.5
Na (mg)	23	150
K (mg)	89	590
Cl (mg)	64	430
Ca (mg)	62	410
P (mg)	31	210
Mg (mg)	7	50
Mn (μ g)	8	50
Se (μ g)	2	13
Vitamin A (μ g RE)	105	700
Vitamin D (μ g)	1.5	10
Vitamin E (mg TE)	0.8	5.4
Vitamin K1 (μ g)	8	54
Vitamin C (mg)	10	67
Vitamin B1 (mg)	0.07	0.47
Vitamin B2 (mg)	0.15	1.0
Niacin (mg)	1	6.7
Vitamin B6 (mg)	0.075	0.50
Folic acid (μ g)	9	60
Pantothenic acid (mg)	0.45	3
Vitamin B12 (μ g)	0.3	2
Biotin (μ g)	2.2	15
Choline (mg)	10	67
Fe (mg)	1.2	8
I (μ g)	15	100
Cu (mg)	0.06	0.4
Zn (mg)	0.75	5

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The product will contain the additional ingredients:
 Bifidobacterium Lactis (BL) : 2×10^7 /gram of dry product

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Claims

1. Infant formula comprising at least one LC-PUFA and at least one probiotic.
- 5 2. Infant formula according to claim 1 wherein the LC-PUFA comprises DHA, in combination or not with ARA.
3. Infant formula according to claim 1 or claim 2, wherein at least one of the probiotics is a Bifidobacteria or a Lactobacillus, preferably *Bifidobacterium lactis*.
- 10 4. Infant formula according to one of claims 1 to 3 wherein at least one of the probiotics is *Streptococcus thermophilus*.
5. Infant formula according to one of claims 1 to 4 further comprising proteins, at least 40% of them being modified sweet whey proteins with no CGMP or reduced CGMP.
- 15 6. Infant formula according to claim 5 wherein the whey proteins with reduced CGMP represent at least 60% of the total proteins, preferably at least 70% of the total proteins.
- 20 7. Infant formula according to claim 5 or claim 6 wherein the proteins are present in a maximum proportion of 2g/100 kcal, preferably 1.85, most preferably between 1.8 and 1.85 g/100 kcal.
- 25 8. Method for strengthening natural immune defenses of an infant or a baby consisting in fully or partly feeding said infant or baby with a formula comprising at least one probiotic and at least one LC-PUFA.
9. Method for strengthening natural immune defenses according to claim 9 with a formula according to any of claims 1 to 7.
- 30 10. Method for strengthening natural immune defenses according to claim 8 or claim 9 by lowering flatulence, vomiting, regurgitation and/or morbidity.
- 35 11. Method for promoting a healthy mental development in an infant or a baby consisting in fully or partly feeding said infant or baby with a formula comprising at least one LC-PUFA associated with proteins and probiotics, wherein at least 40% of the proteins are modified sweet whey proteins comprising no CGMP or reduced CGMP.

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12. Method for promoting a healthy mental development according to claim 11 with a formula according to any of claims 1 to 7.

5 14. Method according to claim 11 or claim 12, wherein the infant or baby is pre-term.

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Abstract

The formula of the invention, intended both for infants and young children, comprises at least one LC-PUFA and at least one probiotic.

- 5 The invention also pertains to methods for strengthening natural immune defenses and method for promoting a healthy mental development in infants or young children by fully or partly feeding them with the afore-mentioned formula.

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